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THE EFFECT OF ATROPINE ON SPONTANEOUS CONTRACTIONS IN BLADDER STRIPS FROM RATS; EFFECT OF THE MUCOSA AND BLADDER OUTLET OBSTRUCTION

Hypothesis / aims of study
Interest in the cholinergic regulation of spontaneous contractions (SCs) has been stimulated by the hypothesis that the key symptom of overactive bladder syndrome, urgency, may be associated with enhancement of SCs by small amount of endogenous acetylcholine released from the mucosa and intramural nerve. There has been a controversy in the effect of atropine on SCs in bladder strips. We have previously reported that a substance released from the mucosa activates SCs in bladder strips of rats and the effect of this substance is pronounced by bladder outlet obstruction (BOO) and partly involves muscarinic receptors in the mucosa. These findings imply that endogenous acetylcholine released from the mucosa can be involved in the generation of SCs in rat bladder strips and its effect is pronounced by BOO. The aim of this study was to examine the difference in the effect of atropine on SCs not only between bladder strips with and without the mucosa but between bladders with and without BOO. The amount of ATP released from bladder strips was also estimated because it has been known that the antimuscarinics inhibit the release of ATP from the urothelium of bladders.

Study design, materials and methods
Female Wistar rats (8 weeks old) were used in this study. The rats were divided into two groups; BOO group and sham-operation group. BOO was induced by incomplete urethral ligation (n=9). Sham-operated rats underwent only the dissection of urethra (n=11). Four weeks following the operation, bladders were removed and weighed. Two pairs of mucosa-intact and –denuded strips from each bladder body were mounted in tissue baths, equilibrated at 1 g resting tension for at least 1 hour and washed with Krebs solution every 20 minutes. After SCs developed, the frequency and amplitude of SCs were recorded, and then for one pair of strips the solution was exchanged to Krebs solution containing 1μM atropine, for the other pair the solution was exchanged to Krebs solution containing vehicle (0.01% DMSO). The bladder strips were incubated in Krebs solution containing atropine or vehicle for 60 minutes, washing every 20 minutes with the fresh solution. The frequency and amplitude of SCs were recorded in each of the periods of 20 minutes between washing, i.e. the first, second and last period of exposure to atropine or vehicle. The solution in tissue baths were taken for the measurement of ATP just before the exposure to atropine or vehicle (baseline) and just before the completion of the last period of exposure to atropine or vehicle. ATP was measured with the luciferin-luciferase assay. The repeated ANOVA followed by Dunnett's post-hoc test and a paired Student's t-test were used for statistical analysis, with a p-value of <0.05 considered statistically significant.

Results
1) The frequency of SCs was not decreased by atropine in mucosa-intact strips from BOO and sham-operated rats. The amplitude of SCs was decreased by atropine both in the mucosa-intact strips from BOO and sham-operated rats when compared to vehicle ($p<0.05$ for both in the first period of exposure to atropine) although the extent of decrease was small; the lowest value of amplitude of SCs over all periods of exposure to atropine was 85% and 92% of baseline value, respectively for BOO and sham-operated rat bladders.
2) The frequency of SCs was significantly decreased by atropine in mucosa-denuded strips from BOO and sham-operated rats ($p<0.05$ for each); the lowest value of frequency of SCs over all periods of exposure to atropine was 89% and 93% of baseline value, respectively for BOO and sham-operated rat bladders. The amplitude of SCs was not changed in mucosa-denuded strips from BOO and sham-operated rats.
3) The difference in the effect of atropine on the amplitude of SCs between the strips with and without the mucosa was greater in BOO rat bladders than in sham-operated rat bladders ($p<0.05$ in the last period of exposure to atropine) while the difference in the effect of atropine on the frequency of SCs between the strips with and without the mucosa was not different between BOO and sham-operated bladders.
4) The released amount of ATP from the mucosa-intact strips was significantly higher than that from the mucosa-denuded strips from both BOO and sham-operated rats ($p<0.01$ for both). Atropine did not change the released amount of ATP from bladder strips irrespective of the presence or absence of the mucosa.

Interpretation of results
Although the effect of atropine on SCs in the rat bladder strip is small, the present study showed a significant change in SCs by atropine. The effect of atropine seems to be different between the strips with and without the mucosa, i.e. the frequency of SCs was not decreased by atropine in the strips with mucosa, but decreased by atropine in the strips without mucosa; the amplitude of SCs was decreased by atropine in the strips with mucosa, but not decreased by atropine in the strips without mucosa. These findings suggest that the muscarinic receptor in the mucosa is not involved in the pace-making of SCs in the intact strips but involved in the activation of detrusor muscle contraction. As atropine has no direct effect on the amplitude of SCs in the mucosa-denuded strip, the activation of detrusor muscle by the mucosa is not due to acetylcholine released from the mucosa. A mucosa-derived substance other than acetylcholine may activate detrusor muscle. ATP does not seem to be this substance as the released amount of ATP from the intact strips was not decreased by atropine. Therefore, a mucosa-derived substance other than acetylcholine and ATP may activate detrusor muscle and the muscarinic receptor in the mucosa is involved in the production and/or the release from the mucosa of this substance. The difference in the effect of atropine on the amplitude of SCs between the strips with and without the mucosa was greater in bladders from BOO rat than in those from sham-operated rat, so it was conformed again that the effect of this mucosa-derived substance is pronounced by BOO.
When the mucosa is absent, the muscarinic receptor in the detrusor muscle layer may be involved in the regulation of the frequency of SCs.

Concluding message

Although the effect of atropine on SCs in the rat bladder strip is small, there is a significant change in SCs by atropine. The effect of atropine seems to be different between the strips with and without the mucosa. A mucosa-derived substance other than acetylcholine and ATP may activate SCs and the muscarinic receptor in the mucosa is involved in the production and/or release from the mucosa of this substance.

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